

Determination of SS31 concentration in main organs of AKI mice

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ROS-responsive chitosan-SS31 prodrug for AKI therapy via rapid distribution in the kidney and long-term retention in the renal tubule

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Detailed protocol

- 1 The ICR mice subjected to renal IR were divided into (i) SS31 group, (ii) C-TK-SS31 group, and (iii) SC-TK-SS31 group.
- 2 At 4 hours after injection of formulations at a dose of SS31 (2 mg/kg), mice were euthanized, and main organs (kidney, heart, lung, liver, and spleen) were harvested.
- 3 Treatment of tissue samples:
 - 1) 10% Tissue homogenate. The tissue blocks were rinsed in cold normal saline to remove blood, dried with filter paper, weighed and put into 5 ml centrifuge tube. The total volume of pre cooled PBS should be 9 times of the weight of tissue block. Use an ophthalmic scissors to cut up the tissue mass as soon as possible. 10% tissue homogenate (homogenizing 10 s at a time, 30 s intermittently, 3-5 times) was prepared by the internal tissue homogenizer in the ice-water bath. The prepared 10% tissue homogenate was centrifuged at 4000 rpm for 10 min. Take the supernatant.
 - 2) Deproteinization with CH₃CN. The supernatant above was deproteinized with a 1.5 volume of acetonitrile. The mixture was centrifuged (10 min, 12000 rpm, ambient temperature), and the supernatant was evaporated under a gentle stream of nitrogen.
 - 3) Reconstitute. The deproteinized residue was reconstituted in 100 mM H₂O₂ to concentrate five times. Suspend for 30s and let stand for 10 min. The mixture was centrifuged (10 min, 12000 rpm, ambient temperature). Take the supernatant.
 - 4) Determination by HPLC. The supernatant was determined by HPLC. HPLC conditions: SS31 was quantified by HPLC with a C18 column. Acetonitrile/water with 0.1% TFA (25:75, v/v) was used as the mobile phase. The column temperature and the detection wavelength were 25°C and 220 nm with a flow rate at 1.0 ml/min and an injection volume of 20 µl.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Du, Y. (2020). Determination of SS31 concentration in main organs of AKI mice. Bio-protocol Preprint. bio-protocol.org/prep637.
2. Liu, D., Shu, G., Jin, F., Qi, J., Xu, X., Du, Y., Yu, H., Wang, J., Sun, M., You, Y., Zhu, M., Chen, M., Zhu, L., Shen, Q., Ying, X., Lou, X., Jiang, S. and Du, Y. (2020). ROS-responsive chitosan-SS31 prodrug for AKI therapy via rapid distribution in the kidney and long-term retention in the renal tubule. Science Advances 6(41). DOI: [10.1126/sciadv.abb7422](https://doi.org/10.1126/sciadv.abb7422)

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